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Molecular Docking Analysis of Helicobacter Pylori RecAwith Gallic Acid

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ABSTRACT: Medicine plants have a diverse array of metabolites that potentially act as a source of new drug molecules exhibiting antimicrobial activities. One of promising molecule is gallic acid, a phenolic compound found abundantly in green tea, banana, strawberries, lemon etc. Numerous studies have shown beneficial effect of gallic acid, as it exhibits multiple positive effects in humans such as maintaining intestinal health by controlling gut microflora, immune response and has a role in DNA Damage response. RecA protein, a recombinase protein, is a central molecule in DNA damage repair pathway and shown to be inhibited by gallic acid. However, it is still an open question how gallic acid is inhibiting H. pyloriRecAprotein.In this present study we have tried to find out the amino acid residues involved in the interaction with drug molecule i.e gallic acid by employing in silico molecular docking analysis. The result indicates that gallic acid bind to H. pyloriRecAwalker A motif also known as P-loop. Our finding will pave the way for future designing of drug molecule or analogues for improved efficacy.

Keywords: Homologous modelling;H. pylori; RecA; gallic acid; drug molecule, DNA repair

I. INTRODUCTION

Helicobacter pylori is a gram-negative bacterium known for its clinical significance and ability to cause infections particularly in gastrointestinal system. H. pylori infection is attributed to stomach ulcers in up to 80% of cases and in duodenal ulcerin approximately 90% of the cases Most of the H pylori can be treated with Clarithromycin along with combination with other drugs[1].

The adaptability and success of H. pylori as a pathogen are linked to its genomic plasticity. A key contributor to this plasticity is the DNA damage, repair and recombination system present in the bacteria. The recombination system facilitates the exchange of genetic material, leading to genetic diversity and evolution. HR also felicitate adaptation in response to abiotic and biotic stress which includes antibiotic exposure stress[2]. However,with the bacteria acquiring multi drug resistance, discovery, isolationand characterization of novel drug moleculeformsmedicinal plants offer a glimpse of hope. One such molecule is gallic acid found green tea, banana, strawberries, lemon etc and.Gallic acid inhibit bacterial biofilm formation, bacterial virulence factor and preventing bacterial host receptor interactionand promote lipid peroxidation [3]

RecA, a highly conserved molecule is implicated in DNA damage, repair and homologous repair pathway exhibit multiple biochemical activities such as DNA dependent ATPase, ssDNA binding, role in LexA cleavage and strand exchange reaction. In the presence of ATP molecule, RecA forms a long nucleoprotein filament by binding to ssDNA. This nucleoprotein filament causes auto cleavage of a transcriptional regulator LexA protein, responsible for expression of SOS genes[4,5].Gallic has also been implicated to target protein involved in DNA [3].

In this present study, effort was made to identify the potential amino acid of HpRecA, which may interact with gallic acid as this interaction may be responsible for the inhibtion of RecA mediated biochemical activities in bacteria. The study was performed considering the highly conserved nature of RecA protein and its domain to deduce the amino acid involved in interaction using Autodock Vina.The results of this study will help to better understand the molecular, biochemical and functional activities of RecA protein.

II. MATERIAL AND METHOD

Sequence alignment ofE. coli RecA and H. pyloriRecA

The amino acid sequence of E. coli RecA and H. pyloriRecA was downloaded in FASTA format from Uniprot data base

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(https://www.uniprot.org/). Sequence alignment of E. coli RecA and H. pyloriRecAwas performed in Clustal Omega (https://www.ebi.ac.uk/jdispatcher/msa/clustalo)[6] and the results were analysed and interpreted using Jalview 2.11.3.3.

Targeted H. pyloriRecA protein

In the absence of crystal structure of H. pyloriRecA, homology modelling was performed using Swiss model (https://swissmodel.expasy.org/). Experimentally deduced closest structure model was chosen to build the homology model. The resulting model was downloadedand optimized for molecular docking analysis by adding hydrogen polar charges, Kollman charge,further converted to.pdbqt format using Autodock4 tools.

Ligand Preparation:

The chemical structures for the natural phenolic compound Gallic acid 3,4,5-trihydroxybenzoic acid which was found in the extracts of pomegranates, walnuts, bananas, strawberries.were retrieved from Pubchem database These downloaded files were optimised for the molecular docking and subsequently converted to pdbqt format using Autodock tools 4.2.6. The downloaded file .sdf was converted to .pdb format using open Babel. Drug structure was further optimised for docking analysis and converted to .pdbqt format using Autodock4 tools.

Molecular docking analysis of SaRecA and gallic acid

To deduce the binding mode of RecA with drug molecule, docking analysis was performed by using Auto dock vina. The 3D structure of gallic acid and H. pylori RecA protein were applied in pdbqt format. Since, the exact active site is not known the entire protein molecule was selected leaving the flexible c-terminal tail. The size of the grid was kept at 100 X 100 X 100Å and the grid spacing was 0.500 Å. Docking simulations were done using Autodock Vina. 10 conformers (run) were generated for drug during the docking phase.After the docking was completed, the lowest free energy values of binding were chosen for further analysis. [7,8].

III. RESULT AND DISCUSSION

Result 1. Sequence alignment

Sequence alignment of EcRecA and HpRecA amino acid sequence sequences revealed 60.76 % sequence identity. Interestingly, the alignment shows that the two protein share conserved motifs and domains such as walker A and walker B motif along the entire streatch of the protein. Sequence alignment also revealed the highly conserved N-terminal, core domain and C-terminal domain, which further strengthen the Swiss Model predicted 3D structure[fig 1].

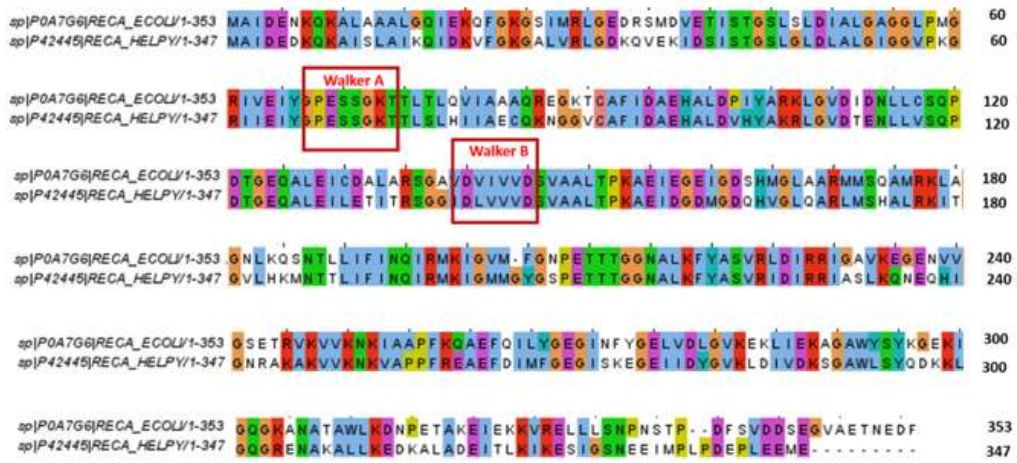

Figure 1. **Pairwise Sequence alignment of RecA from different bacterial species**.

Amino acid in FASTA format were aligned using the Clustal Omega and displayed by Jalview. The positions of the amino acids are indicated on the right of the figure. The conserved amino acid residues are shaded as follows: blue, acidic residues ; red, hydrophobic residues;

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magenta, basic residues; and green, hydroxyl/ amines/glutamine. conserved motifs are enclosed in a box.The details of the accession number of amino acid sequence used for the multiple sequence alignment are: P0A7G6-Escherichia coli (strain K-12) RecA; P42445- H. pylori RecA

Result 2. Molecular docking analysis

In the present study, interaction between H. pylori RecA,protein and gallic acid was analysed using Autodock Vina. In the absence of crystal structure of H. pylori RecA, Swiss Model was used to generate the 3D model of protein based on homology modelling. (fig 2). The 3D organization of H. pylori RecA was a similar to most of RecA protein characterized till now, confirming the highly conserved nature of the protein.

3DGallic acid structure was accessed from pubchem and processed using Open Babel and Autodock4.The structure was saved as .pdb file (Fig.3). Molecular docking was performed between H. pylori RecAand gallic acid using Autodock Vina and the pose with lowest binding energy was chosen for further analysis (fig. 4). Molecular docking was performed and out of all the poses, pose 1 was found to be of lowest binding energy (-5.7 kcal/mol) at 2 Å.A total of 08 amino acid residues were found to be in the close proximity of active site ofH. pylori RecA. Interestingly, amino acid Lys 73 was found be directly interacting with gallic acid in our docking studies (fig. 5, 6)

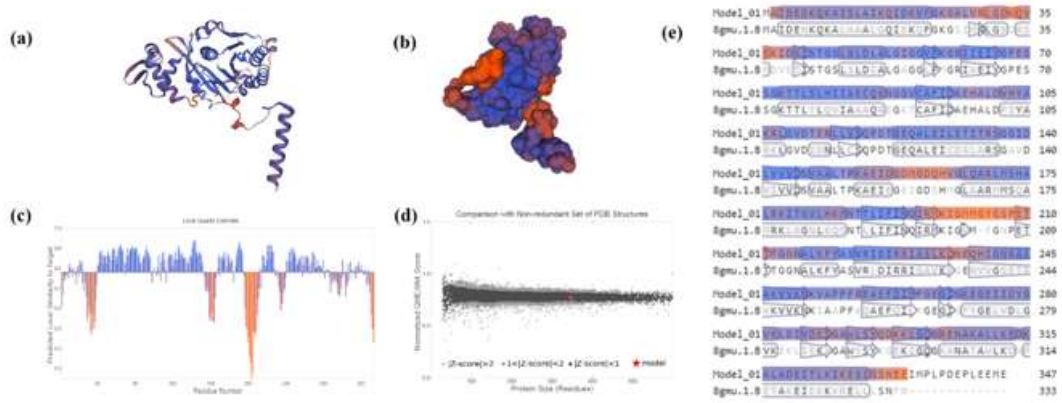
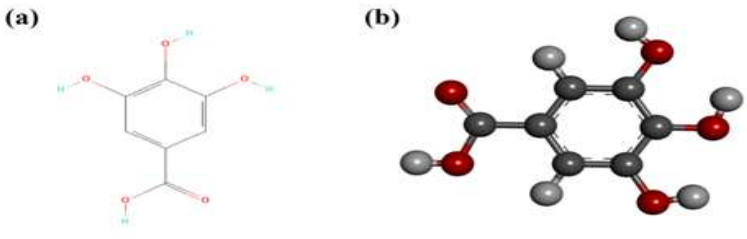


Fig: 2. **Generation of 3D model using Swiss Model**: (a) and (b) showing the ribbon and space filling 3D model of HpRecA, (c) displaying the quality estimate (d) showing non redundant set of PDB structure (red star mark should always be inside) (e) showing the sequence alignment used as template by Swiss Model



(a) 2-D structure of gallic acid (b) 3-D structure of gallic acid

Figure 3. **Structure of gallic acid:** The drug (2-D and 3-D) of gallic was accessed from Pubchem and optimized using Open Babel and Autodock4.

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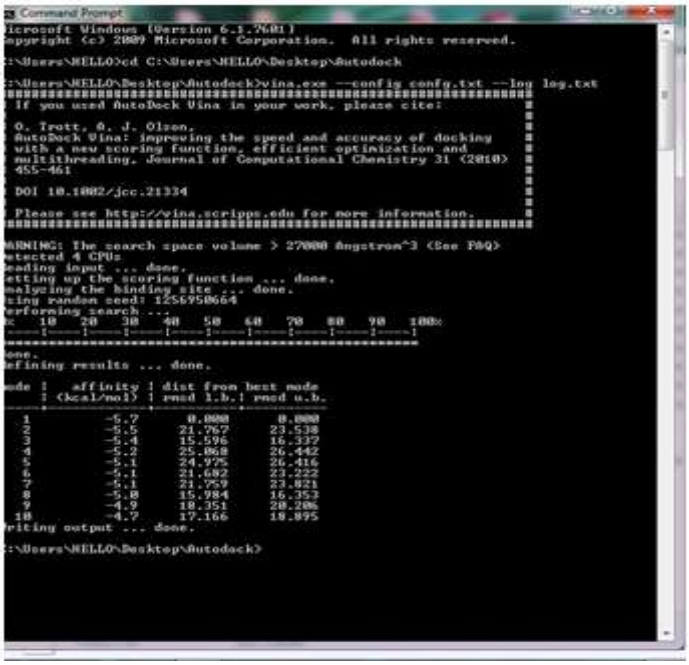

Fig: 4**Autodock Vina result**: all the ten runs were seen with the number 1 showing the least binding energy

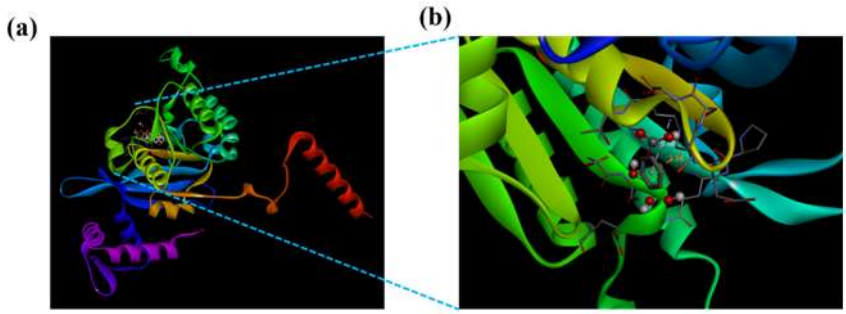

Fig: 5Molecular interaction of gallic acid and HpRecA.: The docking results shows the gallic acid interacting with HpRecA at the active site

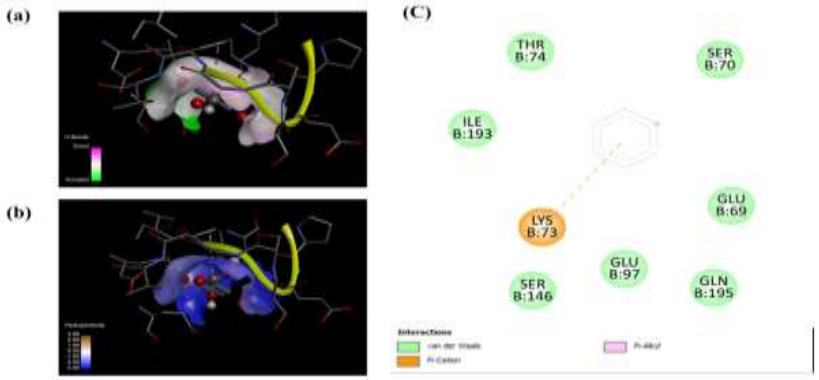

Fig: 6**HpRecAamino acid interaction with gallic acid,** (a) Hydrogen bond interation spread (b) Hydrophibicity spread (c) active site amino acid

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IV. CONCLUSION

Molecular docking has become a very important tool in identifying novel drug targets in protein molecule andalso helps in predicting the protein active site and interacting amino acid residue with drug molecule.

In this study, possible interacting amino acids of HpRecA was identified by molecular docking by observing and analysing interaction with the drug molecule gallic acid. Interestingly, amino acid Lys 73 was found to be interacting with the drug gallic acid. This amino acid is present in walker A motif, also known as P-loop and is responsible for ATP binding, indicating that gallic acid might compete with ATP molecule. Absence of ATP binding and hydrolysis will inhibit all the downstream reaction thus inhibiting the recombination pathway. Based on the study, it would be interesting to further analyse the role of lys 73 amino acid and effect of site directed mutation on the interaction of HpRecA and gallic acid.

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